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14. ABSTRACT

An understanding of the identity of the target cell for prostate cancer development is essential to the development of accurate diagnostic, prognostic and therapeutic approaches. We hypothesized that that loss of the prostate tumor suppressor Nkx3.1 sensitizes cells to prostate tumorigenesis by arresting cells in a precursor transit amplifying state that is more susceptible to tumorigenesis by oncogenes such as c-MYC that can impart self-renewal potential. Further we hypothesize that Nkx3.1 function is regulated by the pioneer factor FoxA1. Our specific goals were to test the susceptibility of Nkx3.1-null transit amplifying cells to transformation and tumorigenicity in response to MYC and to test the hypothesis that FOXA1 is cofactor involved in the regulation of a subset of NKX3.1 target genes in prostate cells. We have established a tissue recombination system for prostate regeneration using primary mouse prostate cells. Using this system, we have obtained evidence that loss of Nkx3.1 cooperates with MYC expression in promoting prostate tumorigenesis in vivo. We have also obtained evidence that Nkx3.1 binding sites tend to co-occur with FOXA1 binding sites in prostate cells. Analysis of data genomic NKX3.1 and FOXA1 binding data from LNCaP cells indicated a significant overlap, with binding of these factors occurs near each other on the DNA.

15. SUBJECT TERMS

Prostate cancer, stem cell, Nkx3.1 tumor suppressor, MYC oncogene

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INTRODUCTION

Knowledge of the target cell(s) that initiate prostate cancer is important for the success of efforts aimed at developing efficient diagnostic, prognostic and therapeutic methods (Dirks, 2008; Lawson and Witte, 2007). NKX3.1 encodes a homeodomain transcription factor whose expression is androgen-dependent and largely restricted to the luminal epithelial cells of the prostate (Abate-Shen and Shen, 2000; Abdulkadir, 2005). Functional studies indicate that the primary defect in Nkx3.1 null mice is a failure of transit amplifying cells to undergo terminal differentiation and exit the cell cycle in a timely manner (Abdulkadir et al., 2002; Magee et al., 2003). Loss of Nkx3.1 leads to the dysregulation of many genes, but whether this is a direct or indirect effect is unknown (Magee et al., 2003). We sought to determine if tumor progression of tumorigenic cells initiated by Nkx3.1 loss requires additional oncogenic events such as Myc overexpression which may promote self-renewal. Furthermore, the ability of Nkx3.1 to bind and regulate target genes is likely to be modified by additional factors, including so-called pioneer factors that have the ability to bind nucleosomal DNA and destabilize nucleosomes thereby allowing other transcription factors to access their sites (Zaret, 1999),(Zaret et al., 2008). Our preliminary data suggest that the forkhead protein FOXA1 may be such a factor for Nkx3.1.

BODY

We provide a revised Year 2 Report addressing only Year 2 (1 June 2010-31 May 2011) progress.

Aim 2: To test the hypothesis that FOXA1 is cofactor involved in the regulation of a subset of NKX3.1 target genes in prostate cells. We will determine whether FOXA1 is required as a "pioneer factor" to allow NKX3.1 to bind to chromatin and regulate these target genes. The role of histone modifications, specifically histone H3 methylation on FOXA1 and NKX3.1 binding to target genes will be assessed. Finally, we will examine the functional significance of selected NKX3.1/FOXA1-regulated genes in prostate tumorigenesis. (Modified, approved Aim)

Tasks:
Task 4: Use siRNA to knockdown FOXA1, NKX3.1 or both in LNCaP cells, then examine target gene expression for 20 selected NKX3.1/FOXA1 targets by qRT-PCR. FOXA1. Assess if FOXA1 knockdown globally affects binding of NKX3.1 to chromatin, and vice versa. (Months 13-18) ***Task 5:*** Examine if NKX3.1 interacts with FOXA1 using co-immunoprecipitation. Map interaction domains. Examine interaction on chromatin by ChIP-re-ChIP analysis. (Months 15-24) ***Task 6:*** Examine changes in H3K4me1/2 following FOXA1 or NKX3.1 knockdown. Overexpress the lysine demethylase KDM1, and then assess H3K4me1/2 status and FOXA1 and NKX3.1 binding by ChIP. (Months 18-36) **Task 7:** Determine the correlation between the occurrence of NKX3.1/FOXA1 binding sites within 20Kb of the TSS of genes and the co-expression of the genes with FOXA1 and NKX3.1, using published databases of prostate cancer microarray studies. (Months 18-24)

Results: Tasks 4-7: So far, we have confirmed that both NKX3. And FOXA1 are expressed abundantly in LNCaP cells. We have obtained LNCaP cells with knockdown of NKX3.1. We show interaction between NKX3.1 and FOXA1 by co-immunoprecipitation both when exogenous proteins are expressed in 293 cells and when endogenously expressed proteins are examined in LNCaP cells (Figure 1). Furthermore, interaction occurs even in the presence of ethidium bromide, indicating that binding occurs independent of DNA. We have mapped interaction of NKX3.1 to the forkhead domain of FOXA1 (Figure 2). We have also examined co-occurrence of NKX3.1 and FOXA1 binding across the genome using our own data and publically available databases. The results show a significant overlap between NKX3.1 and FOXA1 sites (Figure 3). The average distance between the sites is only 65 nt, suggesting that these proteins regulate the same genes and may be involved in the same transcriptional complexes.

KEY RESEARCH ACCOMPLISHMENTS

- Demonstration of NKX3.1 and FOXA1 expression in LNCaP cells
- Demonstration of binding between NKX3.1 and FOXA1
- Mapping the NKX3.1 interacting site in FOXA1

- Demonstration of overlap between NKX3.1 and FOXA1 binding sites in LNCaP cells

REPORTABLE OUTCOMES:

None

CONCLUSION:

During this reporting period, we have focused on examining the possible role of FOXA1 as a cofactor in the regulation of NKX3.1 target genes. As a pioneer factor able to remodel nucleosomes and "open" chromatin, FOXA1 may be required to allow NKX3.1 to access its DNA binding sites in chromatin. Consistent with this hypothesis, we have so far shown that NKX3.1 and FOXA1 bind to DNA sites that are very close to each other in the genome. Furthermore, FOXA1 can physically interact with NKX3.1. Ongoing studies are focused on examining the effect of modulating FOXA1 levels on the chromatin state and NKX3.1 binding.

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APPENDICES

None

SUPPORTING DATA

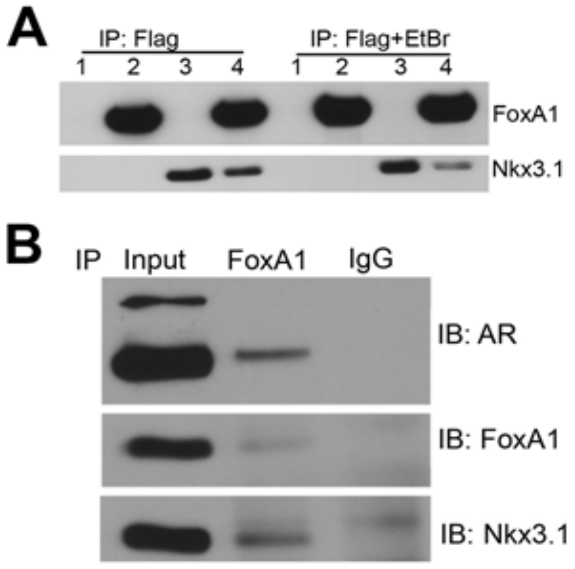

Figure 1: NKX3.1 interacts with FOXA1. A) 293Tcells were transfected with: pcDNA3.1 and plenti/Flag-His empty vector (lane 1); pcDNA3.1 and Flag-His-FoxA1 (lane 2); HA-Nkx3.1 and plenti/Flag-His empty vector (lane 3); HA-Nkx3.1 and Flag-His-FoxA1 (lane 4). All cell lysates were immunoprecipitated by an anti-Flag M2 affinity gel in the absence or presence of ethidium bromide (EtBr). Western blot was performed using individual antibodies as indicated. **B**) LNCaP cell lysates were

immunoprecipitated with Foxa1 or corresponding goat IgG as control. Western blot was performed using individual antibodies as indicated. AR was included as a positive control as it is known to interact with FOXA1.

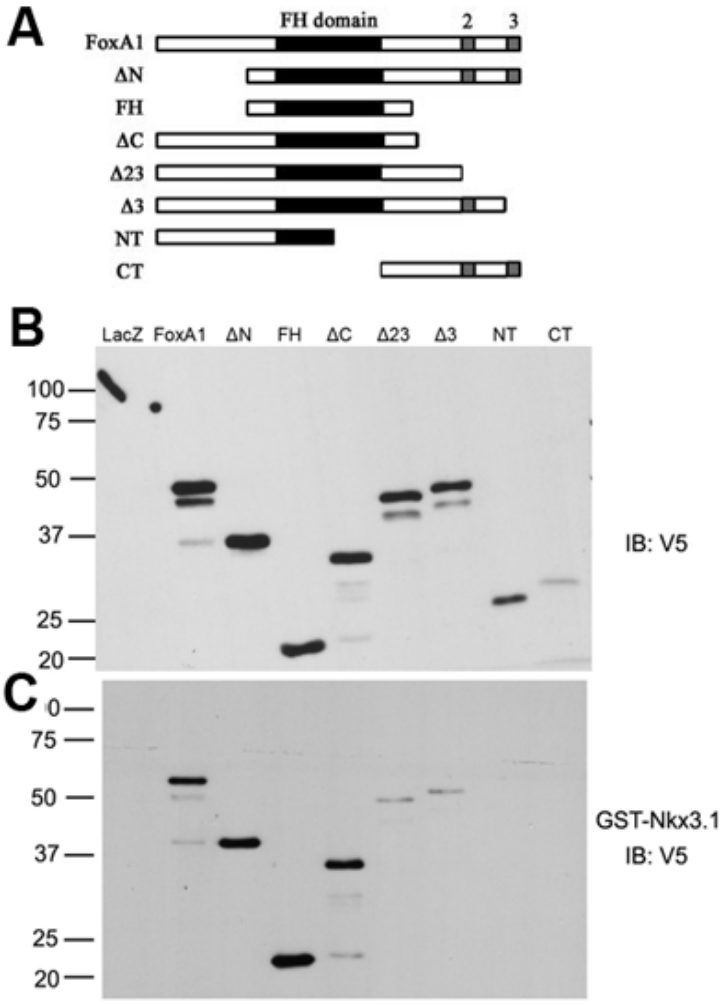

Figure 2. NKX3.1 interaction with FOXA1 depends on the forkhead (FH) domain. A) Schematic of FOXA1 deletion mutants used in GST pull-down. **B**) Expression of eight V5-tagged FOXA1deletions as well as LacZ (negative control) after in vitro translation. **C**) The in vitro translated proteins were incubated with GST-Nkx3.1 and pull down assays performed to determine the NKX3.1-interacting region in FOXA1.

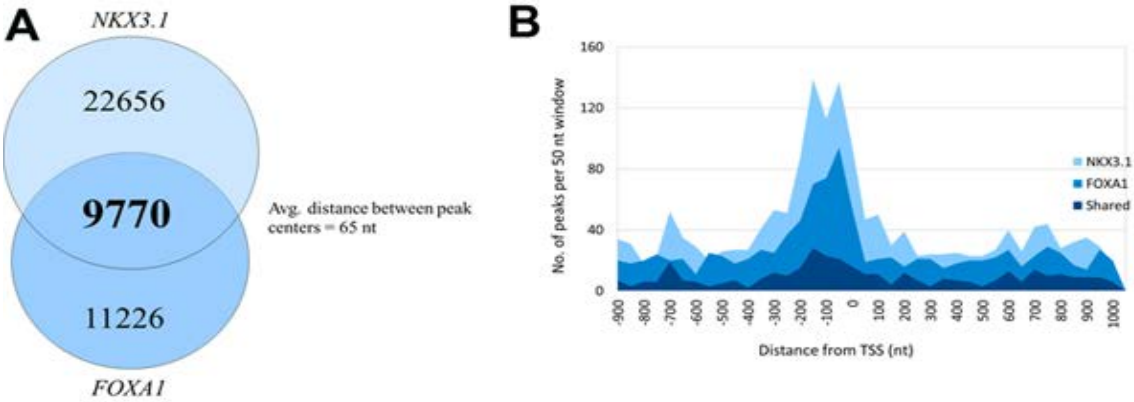

Fig. 3 NKX3.1 and FOXA1 binding sites overlap in LNCaP cells. A) NKX3.1 and FOXA1 binding site s(peaks) were obtained from ChIP-Seq analysis in LNCaP cells. The Venn diagram shows overlap of these binding sites within 300 nt. The average distance between peaks is 65nt. B) Distribution of NKX3.1 peaks, FOXA1 peaks or peaks shared by NKX3.1 and FOXA1 relative to transcription start sites of genes in LNCaP cells.